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Method of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group—Determination of Triazine and Chloroacetanilide Herbicides in Water by Solid-Phase Extraction and Capillary-Column Gas Chromatography/Mass Spectrometry With Selected-Ion Monitoring

By L.R. ZIMMERMAN and E.M. THURMAN

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For additional information write to:

District Chief
U.S. Geological Survey
4821 Quail Crest Place
Lawrence, KS 66049–3839

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Conversion Factors

Multiply	By	To obtain
ounce (oz)	0.02957	liter
pound (lb)	453.6	gram
pound per square inch (lb/in^2)	6.895	kilopascal

Temperature can be converted to degrees Celsius (°C) or degrees Fahrenheit (°F) by the equations:

$$°C = 5/9 (°F - 32)$$
$$°F = 9/5 (°C) + 32.$$

Miscellaneous Abbreviations

degrees Celsius per minute (°C /min)
gram (g)
inside diameter (i.d.)
mass to charge (m/z)
meter (m)
micrometer (µm)
milligram (mg)
millimeter (mm)
millisecond (ms)
minute (min)
nanogram (ng)

Abbreviated Water-Quality Units

liter (L)
microgram per liter (µg/L)
microgram per milliliter (ug/mL)
microliter (µL)
milligram per milliliter (mg/mL)
milliliter (mL)
milliliter per minute (mL/min)
nanogram per microliter (ng/µL)

Method of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group—Determination Triazine and Chloroacetanilide Herbicides in Water by Solid-Phase Extraction and Capillary-Column Gas Chromatography/Mass Spectrometry With Selected-Ion Monitoring

By L.R. Zimmerman[1] *and* E.M. Thurman[2]

Abstract

A method for the isolation and analysis of 13 triazine and chloroacetanilide herbicides and 3 herbicide metabolites in natural-water samples is described. The herbicides and metabolites are isolated by octadecyl (C-18) solid-phase extraction and determined by capillary-column gas chromatography/ mass spectrometry with selected-ion mode monitoring. Water samples are filtered to remove suspended particulate matter and then are pumped through disposable solid-phase extraction columns containing octadecyl-bonded porous silica to extract the compounds. The adsorbed herbicides and metabolites are removed from the columns by elution with ethyl acetate. Extracted herbicides and metabolites are determined by capillary-column gas chromatography/mass spectrometry with selected-ion monitoring of two to three characteristic ions. The upper concentration limit for the method is 5 micrograms per liter without dilution. Method detection limits range from 0.03 to 0.05 microgram per liter in reagent-, surface-, and ground-water samples. Mean recovery in reagent-water samples is 96.8 percent.

INTRODUCTION

Approximately three-fourths of all pre-emergent herbicides in the United States are applied to row crops in a 10-State area of the Midwestern United States, where herbicides frequently are detected in surface water (Gianessi and Puffer, 1991; Thurman and others, 1991). Because many herbicides and their metabolites are water soluble, they may leach into ground water (Hallberg, 1989; Thurman and others, 1991; Kolpin and others, 1993) as well as be transported in surface runoff (Wauchope, 1978; Leonard, 1988). Reconnaissance studies in the Midwest have shown widespread detection of herbicides, such as atrazine and its metabolites, in surface water (Wauchope, 1978; Leonard, 1988; Thurman and others, 1991, 1992) and in ground water (Hallberg, 1989). Furthermore, two metabolites of atrazine, deethylatrazine and deisopropylatrazine, also are detected frequently in basins throughout the Midwestern United States, with both atrazine and cyanazine as source compounds (Thurman and others, 1994).

This report describes a method of analysis for determination of two classes of herbicides, triazine and chloroacetanilide, in natural-water samples. The method was developed by the U.S. Geological Survey (USGS) Organic Geochemistry Research Group in

[1]University of Kansas, Center for Research, Inc., Lawrence, Kansas.

[2]U.S. Geological Survey, Lawrence, Kansas.

Lawrence, Kansas (Thurman and others, 1992; Meyer and others, 1993). The method combines octadecyl (C-18) solid-phase extraction (SPE) for isolation and gas chromatography/mass spectrometry (GC/MS) operated in the selected-ion monitoring (SIM) mode for selective confirmation and quantitation of the herbicides. This method supplements other methods of the USGS and was implemented by the Organic Geochemistry Research Group in 1989.

The method of analysis described in this report has been assigned the method code "O-2132-99." This unique code represents the automated method of analysis for organic compounds as it is described in this report and can be used to identify the method.

This report provides a detailed description of the method, including the apparatus, reagents, instrument calibration, and the SPE procedure required for sample analysis. Estimated method detection limits, extraction recoveries, and percent variances for 13 triazine and chloroacetanilide herbicides and 3 metabolites are presented.

METHOD OF ANALYSIS (O-2132-99)

Scope and Application

This method is suitable for the determination of low concentrations (in micrograms per liter) of triazine and chloroacetanilide herbicides and metabolites in natural-water samples (table 1). This method is applicable to herbicides and metabolites that are (1) efficiently partitioned from the water phase onto an octadecyl (C-18) silica phase that is chemically bonded to a solid silica matrix and (2) sufficiently volatile and thermally stable for gas chromatography. Suspended particulate matter is removed from the samples by filtration, so this method is suitable only for dissolved-phase herbicides and metabolites.

Compounds were selected because of their extensive use in the United States and their importance to current (1998) studies being conducted by the USGS. The calibration range for the method is equivalent to concentrations from 0.05 to 5.0 µg/L without dilution.

Table 1. Herbicide and metabolite compounds suitable for determination using method described, with registry numbers and molecular weights

[CAS, Chemical Abstract Service; AMID, chloroacetanilide; MET, metabolite; TRI, triazine; --, not available]

Compound	Class	CAS registry number	Molecular weight (atomic mass units)
Acetochlor	AMID	34256–82–1	269
Alachlor	do.	15972–60–8	269
Ametryn	TRI	834–12–8	227
Atrazine	do.	1912–24–9	215
Cyanazine	do.	21725–46–2	240
Cyanazine amide	MET	--	258
Deethylatrazine	do.	6190–65–4	187
Deisopropylatrazine	do.	1007–28–9	173
Metolachlor	AMID	51218–45–2	283
Metribuzin	TRI	21087–64–9	214
Prometon	do.	1610–18–0	225
Prometryn	do.	7287–19–6	241
Propachlor	AMID	1918–16–7	211
Propazine	TRI	139–40–2	229
Simazine	do.	122–34–9	201
Terbutryn	do.	886–50–0	241

Summary of Method

Natural-water samples are filtered at the collection site using glass-fiber filters with 0.7-µm pore diameter to remove suspended particulate matter. In the laboratory, filtered water samples are passed through a preconditioned C-18 column. The adsorbed compounds are removed from the C-18 with ethyl acetate. The eluant is evaporated further under nitrogen. The sample components are separated, identified, and measured by injecting an aliquot of the concentrated extract into a high-resolution, fused-silica capillary column of a GC/MS system under selected-ion mode (SIM). Compounds eluting from the GC column are identified by comparing their measured ions and retention times to reference ions and retention times obtained by the measurement of control standards under the same conditions used for samples. The concentration of each identified compound is measured by

relating the MS response of the quantitation ion produced by that compound to the MS response of the quantitation ion produced by a compound that is used as an internal standard. Surrogate compounds, whose concentrations are known in every sample, are measured with the same calibration procedure.

Interferences

Organic compounds having identical characteristic ions and GC retention times to those of the herbicides and metabolites of interest may interfere.

Apparatus and Instrumentation

- *Analytical balances*—Capable of accurately weighing 0.0100 g ±0.0001 g.
- *Autopipettes*—10-, 100-, and 200-µL, variable-volume autopipettes with disposable tips (Rainin, Woburn, MA, or equivalent).
- *Millilab 1A workstation*—Automated SPE workstation with an online computer. The two syringe pumps on the fluidics module are equipped with a 5- and a 1-mL syringe (Waters, Milford, MA).
  - Multiple Intake Accessories (MIAs): Two MIAs are attached to the 5-mL syringe to increase sample load from 3 to 14.
  - Software: Millilab 1A software, version 3.0 (Waters, Milford, MA).
- *Automated solvent evaporator*—The heat-bath temperature needs to be maintained at 45 °C, and the nitrogen gas pressure at 15 lb/in^2 (Turbovap LV or equivalent, Zymark Inc., Hopkinton, MA).
- *Fused-silica capillary column*—Cross-linked methyl siloxane capillary column (12 m x 0.2 mm i.d., 0.33-µm film thickness) (HP Ultra 1 or equivalent, Hewlett Packard, Wilmington, DE).
- *GC/MS benchtop system*—Hewlett Packard (Wilmington, DE), model 5980 series II Plus or equivalent, GC with autoinjector with a Hewlett Packard, model 5970 or equivalent, MS detector.
  - GC conditions: Oven, 60 °C (hold 2 min), then ramp to 200 °C at 6 °C/min, then 30 °C/min to 250 °C and hold for 2 min; injection port, 210 °C; carrier gas, helium; injection volume, 2 µL, splitless injection.
  - MS conditions: Multiplier, 400 over autotune; detector, 280 °C; dwell time 25 ms; mass ions monitored are listed in table 2 (see section on "Calibration").
- *Moisture sieve and oxygen scrubber for carrier gas.*
- *Data system*—computer and printer compatible with the GC/MS system used.
- *Software*—HP DOS ChemStation Software, 1030A Version C (Hewlett Packard, Wilmington, DE) is used to acquire and store data and for peak integration.

Reagents and Consumable Materials

- *Sample bottles*—baked 4-oz amber glass bottles (Boston round) with Teflon-lined lids.
- *Sample filters*—a 0.70-µm glass-fiber filter (Gilson, Middleton, WI).
- *Reagent water*—generated by purification through activated charcoal filtration and deionization with a high-purity, mixed-bed resin, followed by another activated charcoal filtration and finally distillation in an autostill (Wheaton or equivalent, Millville, NJ).
- *Analytical standards*—Standards of the triazine and chloroacetanilide compounds.
- *SPE columns*—C-18 SEP-PAK Plus, containing 360 mg of 40-µm C-18 bonded-silica packing (Waters, Milford, MA).
- *Disposable snap-cap finish centrifuge tubes*—10 mL (Kimble or equivalent, Vineland, NJ).
- *Disposable borosillicate glass culture tubes*—16 mm by 100 mm (Kimble or equivalent, Vineland, NJ).
- *Solvents*—ethyl acetate [American Chemical Society (ACS) grade] and methanol [high-performance liquid chromatography grade (HPLC)].
- *Gas for evaporation*—nitrogen, ultrapure grade.
- *Pasteur pipettes*—(Kimble or equivalent, Vineland, NJ).
- *0.1-mL autosampler vials*—plastic vial with glass cone insert (Wheaton, Millville, NJ).
- *GC carrier gas*—helium, ultrapure grade.

Sampling Methods, Sample-Collection Equipment, and Cleaning Procedures

Following USGS protocol, surface-water samples are collected with a depth-integrating technique at three or more locations across each stream (Ward and

Harr, 1990). The water samples from each site are composited in a single glass container or Teflon bottle.

Samples are withdrawn from the compositing container and filtered through a 0.70-µm glass-fiber filter using a peristaltic pump. Filters are leached with about 200 mL of sample prior to filtration of sample. The filtrate for analysis is collected in baked 125-mL amber glass bottles with Teflon-lined lids. Samples are chilled immediately and shipped to the laboratory within 3 days of collection. At the laboratory, samples are logged in, assigned identification numbers, and refrigerated at 4 °C until extracted and analyzed.

Standards

- *Stock standard solutions*—Obtain herbicides, metabolites, and surrogate standards as pure materials from commercial vendors or chemical manufacturers. If pure materials are obtained, prepare solutions of 1 mg/mL by accurately weighing, to the nearest 0.001 mg, 50 mg of the pure material in a 50-mL volumetric flask and dilute with methanol. Transfer the stock solutions to clean 2-mL vials and store in a freezer. This solution is stable for about 24 months.
- *Primary fortification standard*—Prepare a 1.23-ng/µL concentration, primary fortification standard solution by combining appropriate volumes of the individual stock solutions in a 1-L volumetric flask. Use adjustable autopipette to dispense an appropriate volume and dilute with methanol. Store at less than 4 °C. This solution is stable for about 24 months.
- *Internal standard solution*—Prepare a solution of phenanthrene-d_{10} in ethyl acetate at a concentration of 0.2 ng/µL. This may be purchased as a 100-µg/mL solution in methylene chloride, and 800 µL are diluted into 4 L of ethyl acetate. Store at less than 4 °C. This solution is stable for about 12 months.
- *Surrogate solution*—Prepare solutions of atrazine-d_5 and terbuthylazine in methanol at concentrations of 1.23 ng/µL. If pure materials are obtained, prepare solutions of 1 mg/mL by accurately weighing, to the nearest 0.001 mg, 50 mg of the pure material in a 50-mL volumetric flask and dilute with methanol. Transfer the stock solutions to clean vials and store in a freezer. This solution is stable for about 24 months.
- *Calibration solutions*—Prepare a series of calibration solutions using the primary fortification standard in water that contains all target herbicides and metabolites at concentrations from 0.05 to 2.0 µg/L (0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 µg/L) and the surrogate standards, atrazine-d_5 and terbuthylazine, at a constant concentration of 1 µg/L.

Gas Chromatograph/Mass Spectrometer Performance

Gas Chromatograph Performance Evaluation

- Gas chromatograph performance is evaluated by peak shape, internal standard response, and by comparison of response factors relative to response factors obtained using a new capillary column and freshly prepared calibration solutions (a standard curve). An example of the separation and peak shape of triazine and chloroacetanilide herbicides and metabolites is shown in a total ion chromatogram of a 1.0-µg/L standard solution in (fig. 1). If peak shape deteriorates or if response factors fail to meet the calibration criteria, the injection liner is changed or maintenance on the capillary column is performed to bring the gas chromatograph into compliance. Part of the inlet end on the capillary column may be removed to restore performance. Specifically, poor peak shape and a loss of response for the herbicides and metabolites susceptible to loss on injection—cyanazine or deisopropylatrazine—indicate a need for immediate action.

Mass Spectrometer Performance Evaluation

- Tune the mass spectrometer before each GC/MS sample set (approximately 43 injections or 3 extract sample sets) using the procedure and software supplied by the manufacturer. Parameters in the tuning software are set to give ±0.15 atomic mass unit resolution at masses 69, 219, and 502 in the spectrum of perfluorotributylamine (PFTBA). With the resolution of the 69 ion at 100-percent abundance, mass 219 ion should be 35 ±20 percent, and mass 502 ion should be greater than 3 percent relative

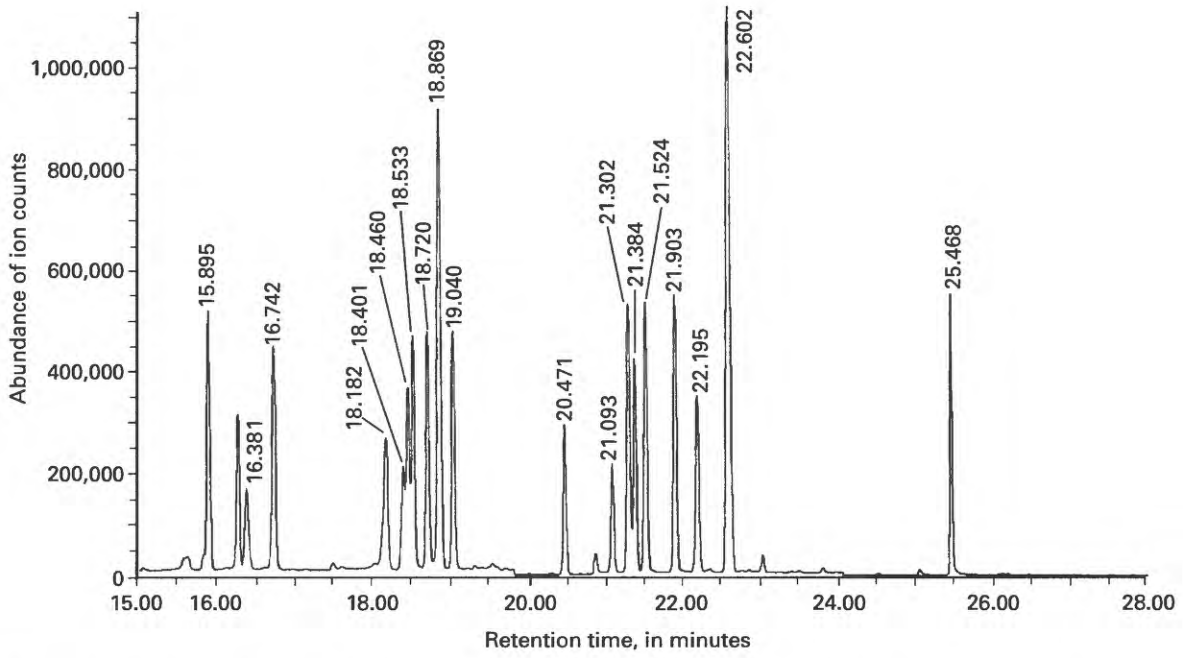

Figure 1. Chromatogram showing total ions of triazine and chloroacetanilide herbicides and metabolites in 1.0-nanogram-per-microliter standard solution. Retention times shown above each peak correspondence to compounds listed in table 1 (see section on "Calibration").

abundance but may vary depending on the mass spectrometer used. Check mass assignments to ensure accuracy to ±0.15 atomic mass units and that mass peak widths measured at one-half the peak height range from about 0.50 to 0.60 atomic mass units.

- Also during the tuning of the mass spectrometer, check the mass spectrometer for the presence of water and air, which indicate leaks in the vacuum. If detected, locate and fix leaks.
- Initially adjust the mass spectrometer to ensure that the established reporting level for each selected compound can be achieved.
- As the first sample in a sample set, inject a solution of pure ethyl acetate to check for contamination.

Calibration

- Acquire initial calibration data by using a new capillary column and freshly prepared calibration solutions. Use these data in the subsequent evaluation of GC/MS performance.
- Acquire data for each calibration solution by injecting 2 µL of each solution into the GC/MS according to the conditions already described. Calculate the relative retention time for each selected compound and the surrogate compounds (RRT_c) in the calibration solution or in a sample as follows:

$$RRT_c = \frac{RT_c}{RT_i}, \quad (1)$$

where RT_c = uncorrected retention time of the quantitation ion of the selected compound or surrogate compound, and

RT_i = uncorrected retention time of the quantitation ion of the internal standard (phenanthrene-d_{10}).

- Initial calibration data are entered into a computer spreadsheet (Microsoft Excel, Microsoft, Inc., Seattle, WA), and ratios are calculated for each quantitation ion relative to the internal standard (phenanthrene-d_{10}). Graphs are made from the GC/MS data by plotting the correlation curve with the phenanthrene-d_{10} ratios of a single ion on the x axis and the concentrations of the standards used on the y axis. Two graphs are made for each ion, one with concentrations ranging from 0 to 0.20 µg/L, and the other with concentrations ranging from 0.20 to 2.0 µg/L. This gives a high and a low curve to keep the response linear. The low curve is plotted with two points at

0. The spreadsheet determines slopes, y intercepts, and correlation coefficient values (r^2) for the graphs.

See table 2 for the respective quantitation ions and internal-standard reference used in these calculations.

- Initial calibration data acquired using a new capillary column and fresh calibration solutions are acceptable if the r^2 value for all curves is greater than or equal to 0.990 for all compounds except cyanazine amide, which needs to have an r^2 value greater than or equal to 0.950.
- Subsequent daily response factors calculated for the majority of compounds need to agree within ±20 percent of the mean response factor for the selected compound of interest. A response factor is equal to the area of the quantitation ion for the selected compound or surrogate divided by the area of the quantitation ion for the internal standard. Analyze at least two calibration standards with each sample set, one high calibration standard ranging from 0.5 to 2.0 µg/L and one low standard ranging from 0.05 to 0.20 µg/L to verify instrument response in each range.

Table 2. Retention times and confirmation ions for triazine and chloroacetanilide herbicides and metabolites analyzed using method described

[min, minute; m/z, mass-to-charge ratio; --, not applicable]

Compound	Retention time (min)	Relative retention time	Quantitation ion (m/z)	Confirmation ion 1 (m/z)	Confirmation ion 1 Percentage of quantitation ion	Confirmation ion 2 (m/z)	Confirmation ion 2 Percentage of quantitation ion
Herbicides or metabolites (in order of increasing retention time)							
Propachlor	15.895	0.843	120	176	33	169	17
Deisopropylatrazine	16.381	.868	173	158	85	145	73
Deethylatrazine	16.742	.887	172	187	32	145	9
Simazine	18.182	.964	201	186	61	--	--
Atrazine	18.460	.978	200	215	60	--	--
Prometon	18.533	.982	210	225	97	--	--
Propazine	18.720	.992	214	229	60	--	--
Metribuzin	20.471	1.085	198	144	15	214	5
Acetochlor	21.093	1.118	223	269	17	--	--
Ametryn	21.302	1.129	227	212	60	--	--
Alachlor	21.384	1.133	188	160	105	--	--
Prometryn	21.524	1.141	241	226	57	--	--
Terbutryn	21.903	1.161	226	241	65	--	--
Cyanazine	22.195	1.176	225	240	56	212	27
Metolachlor	22.602	1.198	162	238	68	240	22
Cyanazine amide	25.468	1.350	214	216	33	132	20
Surrogate compounds							
Atrazine-d_5	18.401	--	205	220	60	--	--
Terbuthylazine	19.040	--	214	229	30	--	--
Internal standard							
Phenanthrene- d_{10}	18.869	1.000	188	--	--	--	--

Procedure

- *Sample preparation*—In the automation of sample extraction, the workstation (Waters Millilab, Milford, MA) requires 23 mL of sample used to prime the pumps and rinse the tubing. Therefore, each sample must be 123 mL, although only 100 mL passes through the SPE column. Conveniently, 123 mL is the exact volume that fits in the body of a 4-oz Boston round bottle. Should an environmental sample contain less than 123 mL, distilled water is added to bring the volume to the required 123 mL. Any volume added is recorded. An extraction sample set consists of nine samples, one duplicate sample, two standard control samples (one high concentration and one low concentration), and two blank control samples. Each bottle is spiked with the surrogate standard solution, terbuthylazine and atrazine-d_5, at concentrations of 1.0 µg/L (100 µL of 1.23-ng/µL surrogate solution into 123 mL) with an autopipette.
- *Workstation preparation*—Before a sample set is extracted on the workstation, each port is flushed with 15 mL of methanol:water (1:1) and then again with distilled water. All SPE columns, test tubes, reagents, working solvents, surrogate spike, and samples then are loaded onto the instrument.
- *Conditioning the SPE columns*—The workstation conditions each SPE column by sequentially passing 1 mL methanol, 1 mL ethyl acetate, 1 mL methanol, and 3 mL distilled water through each column at a flow rate of 20 mL/min by positive pressure.
- *Loading the sample*—Each sample port is flushed with 23 mL of sample, then 100 mL of sample are passed through the SPE column at a flow rate of 20 mL/min.
- *Eluting the SPE column*—Each SPE column is eluted with 3.0 mL ethyl acetate to remove the compounds at a flow rate of 4 mL/min.
- *Spiking of internal standard solution*—After all the samples in a set have been loaded and eluted, the workstation spikes each eluate with 500 µL of 0.2-ng/µL phenanthrene-d_{10} solution.
- *Separation of ethyl acetate and residual water*—Due to the water in the SPE column that is eluted with the ethyl acetate, the workstation pulls the ethyl acetate off the aqueous phase and transfers it into another tube. The inside and outside of the probe are rinsed with clean ethyl acetate between each sample transfer.
- *Evaporation*—The spiked eluant then is evaporated to approximately 75 µL under nitrogen in a water bath at 45 °C.
- *Transfer to vials*—Using a baked disposable Pasteur pipette, the eluant from the 10-mL glass centrifuge tube is withdrawn into a pipette, and transferred to an appropriately labeled GC autosampler vial containing a 0.1-mL insert for GC/MS analysis. The GC autosampler vial is capped and stored at less than 4 °C until analysis by GC/MS.
- *Sample analysis and data evaluation*—Ensure that GC/MS conditions for the analysis of the selected compounds in sample extracts are the same as those used in the analysis of the calibration solutions. Prior to the analysis of any sample extracts, ensure that the PFTBA mass-spectral performance criteria have been met, that the selected-compound calibration data conform to the criteria set forth above, and that the GC/MS is optimized so that the reporting level for each compound can be achieved. Inject 2 µL of the sample extract and acquire data using the GC/MS conditions described in sections.

Calculation of Results

Qualitative Identification

The expected retention time (*RT*) of the GC peak of the quantitation ion for the selected compound of interest needs to be within ±6 seconds of the expected retention time based on the RRT_c obtained from the internal-standard analysis. Calculate the expected retention time as follows:

$$RT = (RRT_c)\,(RT_i)\,, \qquad (2)$$

where RT = expected retention time of the selected compound or surrogate compound,

RRT_c = relative retention time of the selected compound or surrogate compound, and

RT_i = uncorrected retention time of the quantitation ion of the internal standard.

- Mass-spectral verification for each selected compound is done by comparing the relative inte-

grated abundance values of the significant ions monitored with the relative integrated abundance values obtained from the standard control samples. The relative ratios of the ions need to be within ±20 percent of the relative ratios of those obtained in the absence of any obvious interferences.

Quantitation

Calculate the volume of sample processed as follows:

$$DF = \left(\frac{123}{123 - V_{np}}\right)\left(\frac{123}{123 - V_a}\right), \quad (3)$$

where DF = dilution factor,

V_{np} = volume not pumped = 123 - (milliliters not pumped through the SPE column), and

V_a = volume added = (milliliters of reagent water added to a sample that contains less than 123 mL).

The dilution factor is incorporated into the calculation for determining final concentrations of samples.

- If a selected compound has passed the aforementioned qualitative identification criteria, calculate the concentration in the sample as follows:

$$C = \left(\left(\frac{A_c}{A_i}\right)(m) + y\right)(DF), \quad (4)$$

where C = concentration of the selected compound or surrogate compound in the sample, in micrograms per liter;

A_c = area of the quantitation ion for the selected compound or surrogate identified;

A_i = area of the quantitation ion for the internal standard;

m = slope of correlation curve between the selected compound and phenanthrene-d_{10} from the original calibration data;

y = y intercept of correlation curve between the selected compound and phenanthrene-d_{10} from the original calibration data; and

DF = dilution factor as described above.

Reporting of Results

Concentrations of herbicides are reported in micrograms per liter (µg/L) from 0.05 to 5.0. If the concentration is greater than 5.0 µg/L, the sample extract is diluted (volume increased to approximately 150 µL with eluting solvent) and re-analyzed. If the concentration is greater than 10.0 µg/L, the sample is re-extracted with a 1:10 dilution (sample:distilled water) and re-analyzed for those compounds that were greater than 10.0 µg/L.

METHOD PERFORMANCE

A reagent-water sample, a surface-water sample collected from Richie Lake in Isle Royale National Park, Michigan, and a ground-water sample also collected in Isle Royale National Park were used to test the method performance. The surface- and ground-water samples were collected in two 1-L bottles and were split into sixteen 123-mL samples. One set of eight samples was fortified with 0.2 µg/L of each compound, and the other set of eight samples was fortified with 2.0 µg/L of each compound. In addition, unfortified samples of the surface and ground water were extracted and analyzed to determine background concentrations of the pesticides. All subsamples were analyzed in one laboratory (the USGS Organic Geochemistry Research Laboratory in Lawrence, Kansas) using one GC/MS system. Each sample set was extracted and analyzed on different days during March and April 1996, so comparison of different matrices and concentrations included bias from day-to-day variation. Accuracy and precision data from the analyses are listed in tables 3 through 8.

Corrections for background concentrations: Neither surface- nor ground-water samples required correction for background concentrations of compounds. All unfortified reagent-water samples also had no detections.

Method detection limits (MDLs): The MDL is defined as the minimum concentration of a substance that can be identified, measured, and reported with a 99-percent confidence that the compound concentration is greater than zero. MDLs were determined according to procedures outlined by the U.S. Environmental Protection Agency (1992). Eight repli-

Table 3. Mean observed concentration, mean recovery, and percent variance for eight determinations of triazine and chloroacetanilide herbicides and metabolites and surrogate compounds at 0.2 microgram per liter in reagent-water samples

[µg/L, microgram per liter; %, percent; --, not applicable]

Compound	Mean observed concentration (µg/L)	Standard deviation (µg/L)	Mean recovery (%)	Percent variance (%)
Herbicides or metabolites				
Acetochlor	0.23	0.03	115	13
Alachlor	.23	.03	115	13
Ametryn	.24	.03	118	12
Atrazine	.23	.03	115	14
Cyanazine	.21	.02	107	10
Cyanazine amide	.23	.03	113	11
Deethylatrazine	.23	.03	117	13
Deisopropylatrazine	.23	.02	115	10
Metolachlor	.24	.03	119	12
Metribuzin	.27	.04	136	16
Prometon	.24	.03	121	13
Prometryn	.23	.03	115	12
Propachlor	.23	.03	115	12
Propazine	.23	.03	113	13
Simazine	.23	.03	116	13
Terbutryn	.24	.03	120	11
Surrogate compounds				
Atrazine-d_5	--	--	115	13
Terbuthylazine	--	--	116	11

Table 4. Mean observed concentration, mean recovery, and percent variance for eight determinations of triazine and chloroacetanilide herbicides and metabolites and surrogate compounds at 2.0 micrograms per liter in reagent-water samples

[µg/L, microgram per liter; %, percent; --, not applicable]

Compound	Mean observed concentration (µg/L)	Standard deviation (µg/L)	Mean recovery (%)	Percent variance (%)
	Herbicides or metabolites			
Acetochlor	1.90	0.17	95	9
Alachlor	1.91	.18	95	9
Ametryn	1.92	.19	96	10
Atrazine	1.90	.18	95	10
Cyanazine	1.90	.20	95	11
Cyanazine amide	1.70	.25	85	15
Deethylatrazine	1.93	.21	96	11
Deisopropylatrazine	1.71	.23	85	13
Metolachlor	1.95	.18	98	9
Metribuzin	2.01	.21	100	10
Prometon	1.93	.19	96	10
Prometryn	1.90	.19	95	10
Propachlor	1.86	.14	93	8
Propazine	1.92	.20	96	10
Simazine	1.90	.18	95	10
Terbutryn	1.94	.20	97	10
	Surrogate compounds			
Atrazine-d_5	--	--	95	10
Terbuthylazine	--	--	94	10

Table 5. Mean observed concentration, mean recovery, and percent variance for eight determinations of triazine and chloroacetanilide herbicides and metabolites and surrogate compounds at 0.2 microgram per liter in surface-water samples from Lake Richie, Isle Royale National Park, Michigan

[µg/L, microgram per liter; %, percent; --, not applicable]

Compound	Mean observed concentration (µg/L)	Standard deviation (µg/L)	Mean recovery (%)	Percent variance (%)
Herbicides or metabolites				
Acetochlor	0.20	0.03	100	13
Alachlor	.20	.03	100	13
Ametryn	.20	.03	102	15
Atrazine	.21	.03	105	15
Cyanazine	.18	.03	90	16
Cyanazine amide	.22	.02	112	9
Deethylatrazine	.22	.03	111	12
Deisopropylatrazine	.22	.02	108	9
Metolachlor	.21	.03	103	14
Metribuzin	.25	.04	125	16
Prometon	.20	.03	102	16
Prometryn	.20	.03	98	15
Propachlor	.19	.02	97	9
Propazine	.19	.03	97	14
Simazine	.21	.03	103	13
Terbutryn	.21	.03	104	16
Surrogate compounds				
Atrazine-d_5	--	--	103	13
Terbuthylazine	--	--	103	12

Table 6. Mean observed concentration, mean recovery, and percent variance for eight determinations of triazine and chloroacetanilide herbicides and metabolites and surrogate compounds at 2.0 micrograms per liter in surface-water samples from Lake Richie, Isle Royale National Park, Michigan

[µg/L, microgram per liter; %, percent; --, not applicable]

Compound	Mean observed concentration (µg/L)	Standard deviation (µg/L)	Mean recovery (%)	Percent variance (%)
Herbicides or metabolites				
Acetochlor	1.83	0.20	92	11
Alachlor	1.80	.18	90	10
Ametryn	1.82	.20	91	11
Atrazine	1.83	.19	91	10
Cyanazine	1.88	.34	94	18
Cyanazine amide	1.58	.25	79	16
Deethylatrazine	1.74	.18	87	10
Deisopropylatrazine	1.54	.26	77	17
Metolachlor	1.81	.17	90	10
Metribuzin	1.86	.17	93	9
Prometon	1.73	.19	87	11
Prometryn	1.81	.22	90	12
Propachlor	1.76	.15	88	9
Propazine	1.83	.22	91	12
Simazine	1.80	.15	90	9
Terbutryn	1.83	.20	91	11
Surrogate compounds				
Atrazine-d_5	--	--	92	10
Terbuthylazine	--	--	91	11

Table 7. Mean observed concentration, mean recovery, and percent variance for eight determinations of triazine and chloroacetanilide herbicides and metabolites and surrogate compounds at 0.2 microgram per liter in ground-water samples from Isle Royale National Park, Michigan

[µg/L, microgram per liter; %, percent; --, not applicable]

Compound	Mean observed concentration (µg/L)	Standard deviation (µg/L)	Mean recovery (%)	Percent variance (%)
Herbicides or metabolites				
Acetochlor	0.22	0.02	109	11
Alachlor	.21	.02	107	11
Ametryn	.19	.05	97	28
Atrazine	.22	.03	109	13
Cyanazine	.19	.03	95	14
Cyanazine amide	.22	.03	109	15
Deethylatrazine	.22	.03	108	13
Deisopropylatrazine	.20	.03	102	12
Metolachlor	.22	.02	111	10
Metribuzin	.25	.02	123	10
Prometon	.22	.02	110	10
Prometryn	.20	.04	99	21
Propachlor	.21	.03	106	14
Propazine	.21	.02	103	11
Simazine	.21	.02	106	11
Terbutryn	.24	.04	120	18
Surrogate compounds				
Atrazine-d_5	--	--	106	13
Terbuthylazine	--	--	105	12

Table 8. Mean observed concentration, mean recovery, and percent variance for eight determinations of triazine and chloroacetanilide herbicides and metabolites and surrogate compounds at 2.0 micrograms per liter in ground-water samples from Isle Royale National Park, Michigan

[µg/L, microgram per liter; %, percent; --, not applicable]

Compound	Mean observed concentration (µg/L)	Standard deviation (µg/L)	Mean recovery (%)	Percent variance (%)
	Herbicides or metabolites			
Acetochlor	1.74	0.18	87	10
Alachlor	1.69	.17	84	10
Ametryn	1.71	.19	86	11
Atrazine	1.72	.19	86	11
Cyanazine	1.74	.23	87	13
Cyanazine amide	1.64	.21	82	13
Deethylatrazine	1.80	.22	90	12
Deisopropylatrazine	1.61	.24	81	15
Metolachlor	1.74	.21	87	12
Metribuzin	1.88	.22	94	12
Prometon	1.69	.23	84	13
Prometryn	1.70	.19	85	11
Propachlor	1.71	.22	86	13
Propazine	1.73	.19	86	11
Simazine	1.73	.19	86	11
Terbutryn	1.74	.19	87	11
	Surrogate compounds			
Atrazine-d_5	--	--	86	11
Terbuthylazine	--	--	86	10

cate samples of reagent water fortified at 0.05 µg/L were analyzed to determine MDLs (table 9). Each sample was analyzed on different days during May and June 1998, so day-to-day variation is included.

The MDL was calculated using the following equation:

$$MDL = (S)\,(t_{(n-1,\,1-\alpha\,=\,0.99)}), \qquad (5)$$

where S = standard deviation of replicate analysis, in micrograms per liter, at the concentration;

$t_{(n-1,1-\alpha=0.99)}$ = Student's t-value for the 99-percent confidence level with n-1 degrees of freedom (U.S. Environmental Protection Agency, 1992); and

n = number of replicate analyses.

The estimated MDLs ranged from 0.03 to 0.05 µg/L (table 9). According to the U.S. Environmental Protection Agency (1992) procedure, the fortified concentrations should be no more than five times the estimated MDL. The fortified concentrations were within five times the MDL.

Mean recovery: Mean recovery in reagent-, surface-, and ground-water samples was determined by comparing the mean calculated concentration (see "Quantitation" section) from eight replicate samples to the spiked concentration (0.2 µg/L). Recoveries were highest in reagent water at the 0.2-µg/L concentration (table 3) and lowest in ground water at the 2.0-µg/L concentration (table 8). Cyanazine amide and deisopropylatrazine exhibited the lowest recoveries, and metribuzin exhibited the highest recoveries regardless of matrix. The mean recoveries of all compounds in tables 3 and 4 were combined to calculate mean recovery for reagent-water samples. The mean recovery in reagent water at the 0.2- and 2.0-µg/L concentrations is 99.5 percent.

Percent variance: Percent variance in reagent-, surface-, and ground-water samples was determined for all compounds. Percent variances in tables 3, 4, 5, 6, 7, and 8 were combined to calculate the mean percent variance for all matrices. The mean short-term precision is 12.2 percent.

Absolute recovery: Absolute recovery of each compound was determined by comparing eight replicate samples processed using the aforementioned procedure versus solvent spiked with the compounds injected directly into the GC/MS at 1.0-µg/L concentrations. Compound quantitation-ion ratios to internal-standard target-ion ratios were compared. Absolute recoveries are listed in table 10. Absolute recovery is different than mean recoveries listed in tables 3–8 in that mean recoveries are calculated from an initial calibration curve that is processed in the same manner as the samples, thus correcting for routine analyte losses.

Table 9. Method detection limit for eight determinations of triazine and chloroacetanilide compounds at 0.05 microgram per liter in reagent-water samples

[µg/L, microgram per liter; MDL, method detection limit]

Compound	Mean observed concentration (µg/L)	Mean standard deviation (µg/L)	Mean MDL (µg/L)
Acetochlor	0.05	0.02	0.05
Alachlor	.05	.02	.05
Ametryn	.05	.01	.04
Atrazine	.05	.01	.04
Cyanazine	.06	.02	.05
Cyanazine amide	.09	.02	.05
Deethylatrazine	.05	.01	.04
Deisopropylatrazine	.05	.01	.04
Metolachlor	.05	.02	.05
Metribuzin	.05	.01	.04
Prometon	.05	.01	.03
Prometryn	.04	.01	.04
Propachlor	.05	.01	.04
Propazine	.04	.01	.04
Simazine	.05	.01	.04
Terbutryn	.04	.02	.05

CONCLUSIONS

This report presents a method for routine analysis of 13 herbicides and 3 herbicide metabolites in natural-water samples. From the data presented in this report, SPE and determination by GC/MS are shown

Table 10. Absolute recovery for eight determinations of triazine and chloroacetanilide compounds at 1.0 microgram per liter

[%, percent]

Compound	Absolute recovery (%)
Acetochlor	100
Alachlor	101
Ametryn	104
Atrazine	100
Cyanazine	94
Cyanazine amide	93
Deethylatrazine	91
Deisopropylatrazine	57
Metolachlor	100
Metribuzin	103
Prometon	102
Prometryn	105
Propachlor	99
Propazine	103
Simazine	97
Terbutryn	99

to be a sensitive and reliable method for the determination of low concentrations of triazine and chloroacetanilide herbicides and metabolites in natural-water samples. Method detection limits range from 0.03 to 0.05 µg/L. Mean recovery in reagent-water samples is 96.8 percent. The mean percent variance in reagent-, surface-, and ground-water samples for this method is 12.2 percent when fortified at 0.2- and 2.0-µg/L concentrations.

REFERENCES CITED

Gianessi, L.P., and Puffer, C.M., 1991, Herbicides use in the United States—national summary report, revised April 1991: Washington, D.C., Resources for the Future, 128 p.

Hallberg, G.R., 1989, Pesticide pollution in groundwater in the humid United States: Agriculture, Ecosystems, and Environment, v. 26, p. 299–367.

Kolpin, D.W., Burkart, M.R., and Thurman, E.M., 1993, Hydrogeologic, water-quality, and land-use data for the reconnaissance of herbicides and nitrate in near-surface aquifers of the midcontinental United States, 1991: U.S. Geological Survey Open-File Report 93–114, 61 p.

Leonard, R.A., 1988, Herbicides in surface waters, *in* Grover, R., ed., Environmental chemistry of herbicides, volume I: Boca Raton, Florida, CRC Press, p. 45–87.

Meyer, M.T., Mills, M.S., and Thurman, E.M., 1993, Automated solid-phase extraction of herbicides from water for gas chromatographic-mass spectrometric analysis: Journal of Chromatography, v. 629, p. 55–59.

Thurman, E.M., Goolsby, D.A., Meyer, M.T., and Kolpin, D.W., 1991, Herbicides in surface waters of the Midwestern United States—the effect of spring flush: Environmental Science & Technology, v. 25, p. 1794–1796.

Thurman, E.M., Goolsby, D.A., Meyer, M.T., Mills, M.S., Pomes, M.L., and Kolpin, D.W., 1992, A reconnaissance study of herbicides and their metabolites in surface water of the Midwestern United States using immunoassay and gas chromatography/mass spectrometry: Environmental Science & Technology, v. 26, p. 2440–2447.

Thurman, E.M., Meyer, M.T., Mills, M.S., Zimmerman, L.R., Perry, C.A., and Goolsby, D.A., 1994, Formation and transport of deethylatrazine and deisopropylatrazine in surface water: Environmental Science & Technology, v. 28, p. 2267–2277.

U. S. Environmental Protection Agency, 1992, Guidelines establishing test procedures for the analysis of pollutants (App. B, Part 136, Definition and procedures for the determination of the method detection limit): U.S. Code of Federal Regulations, Title 40, Revised as of July 1, 1992, p. 565–567.

Ward, R.D., and Harr, C.A., 1990, Methods for collection and procession of surface-water and bed-material samples for physical and chemical analyses: U.S. Geological Survey Open-File Report 90–140, 71 p.

Wauchope, R.D., 1978, The pesticide content of surface water draining from agricultural fields—a review: Journal of Environmental Quality, v. 7, p. 459–472.

SUPPLEMENTAL INFORMATION

AUTOMATED SOLID-PHASE EXTRACTION PROCEDURE USING MILLILAB 1A WORKSTATION

Millilab Extraction Procedure:

Estimated time for samples : 10 hours
Date : Monday, March 11, 1996

Tube name	Tube type
sample	PORT
elution	TUBE
splspike	TUBE
washprobe	TUBE

Element name	Element type
Sep-Pak+	CARTRIDGE

Port name	Liquid name
Syr1v1m1	sample
Syr1v1m2	sample
Syr1v1m3	sample
Syr1v1m4	sample
Syr1v2m1	sample
Syr1v2m2	sample
Syr1v2m3	sample
Syr1v2m4	sample
Syr1v3m1	sample
Syr1v3m2	sample
Syr1v3m3	sample
Syr1v3m4	sample
Syr1v4m1	sample
Syr1v4m2	sample
Syr1v4m3	distilled1
Syr2v1	distilled2
Syr2v2	Ethyl acetate
Syr2v3	Methanol
Pump name	**Syringe size**
Pump 1	5.0 mL
Pump 2	1.0 mL
Reagent name	**Liquid name**
Reagent 1	Methanol
Reagent 2	Spike
Reagent 3	Not used
Reagent 4	Ethyl acetate

Loop size
4 mL

Technique name		Technique parameters
(1)	WASH PROBE	Solvent = distilled1 Fill Rate = 60 mL/min
		Empty_rate = 60 mL/min Volume = 20 mL Strokes = 4
(2)	SPE SELECT	Cartridge = Sep-Pak+
(3)	SPE LOAD	Working_solvent = distilled1 Rate = 30 mL/min
		Empty_rate = 20 mL/min Volume = 1 mL Level = 0
		From = Methanol To = Waste Gap = 0.1 mL
(4)	SPE LOAD	Working_solvent = distilled1 Rate = 30 mL/min
		Empty_rate = 20 mL/min Volume = 1 mL Level = 0
		From = Ethyl acetate To = Waste Gap = 0.1 mL
(5)	SPE LOAD	Working_solvent = distilled1 Rate = 30 mL/min
		Empty_rate = 20 mL/min Volume = 1 mL Level = 0
		From = Methanol To = Waste Gap = 0.1 mL
(6)	SPE WASH	Solvent = distilled1 Rate = 30 mL/min
		Empty_rate = 20 mL/min Volume = 3 mL To = Waste
(7)	WASH PROBE	Solvent = sample Fill Rate = 60 mL/min
		Empty_rate = 60 mL/min Volume = 15 mL Strokes = 3
(8)	SPE WASH	Solvent = sample Rate = 30 mL/min
		Empty_rate = 20 mL/min Volume = 100 mL To = Waste
(9)	WASH PROBE	Solvent = distilled1 Fill Rate = 60 mL/min
		Empty_rate = 60 mL/min Volume = 10 mL Strokes = 2
(10)	ELEMENT PURGE	Cartridge = Sep-Pak+ Dispose = No Gas = Purge 6 Level = 0
		Clear_time = 0.2 min Purge_time = 1 min To = waste

Technique name		Technique parameters
(11)	SPE LOAD	Working_solvent = Ethyl acetate Rate = 4 mL/min Empty_rate = 4 mL/min Volume = 3.5 mL Level = 0 From = Ethyl Acetate To = Elution Gap = 0.1 mL
(12)	GAS PURGE	Gas = Purge6 To = Waste Level = 0 Clear_time = 0 min Purge_time = 0.4 min
(13)	ELEMENT PURGE	Cartridge = Sep-Pak+ Dispose = No Gas = Purge 6 Level = 0 Clear_time = 0 min Purge_time = 0.3 min To = Elution
(14)	BATCH+PIPETTE	Working_solvent = Ethyl acetate Fill_rate = 4 mL/min Empty_rate = 4 mL/min Asp_level = 60 Disp_level = 560 Volume = 0.5 mL Gap = 0.1 mL From = Spike To = Elution Sample_count = All
(15)	WASH PROBE	Solvent = Ethyl acetate Fill Rate = 6 mL/min Empty_rate = 6 mL/min Volume = 2 mL Strokes = 4
(16)	MIX	Working_solvent = Ethyl acetateFill_rate = 6 mL/min Empty_rate = 6 mL/min Asp_level = 250 Disp_level = 300 Volume = 2.5 mL Gap = 0.1 mL Count = 2 To = Elution
(17)	WASH PROBE	Solvent = Ethyl acetate Fill Rate = 6 mL/min Empty_rate = 6 mL/min Volume = 2 mL Strokes = 4
(18)	PIPETTE	Working_solvent = Ethyl acetate Fill_rate = 4 mL/min Empty_rate = 4 mL/min Asp_level = 240 Disp_level = 550 Volume = 4.0 mL Gap = 0.2 mL From = Elution To = Splspike

Technique name		Technique parameters
(19)	MIX	Working_solvent = Ethyl acetate Fill_rate = 6 mL/min Empty_rate = 6 mL/min Asp_level = 150 Disp_level = 150 Volume = 3.0 mL Gap = 0.1 mL Count = 2 To = Wash_probe
(20)	GAS PURGE	Gas = Purge6 To = Waste Level = 0 Clear_time = 0 min Purge_time = 0.3 min
(21)	BUBBLE MIX	Gas = Purge6 To = Washprobe Level = 0 Clear_time = 0 min Purge_time = 0.3 min
(22)	WASH PROBE	Solvent = Ethyl acetate Fill Rate = 6 mL/min Empty_rate = 6 mL/min Volume = 1 mL Strokes = 2
(23)	SPE DONE	Dispose = No